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PROTEINS (ANTIBODIES) WHICH REACT SPECIFICALLY WITH DNA DAMAGED BY ULTRAVIOLET RADIATION

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ABSTRACT. Antibodies which react with UV-irradiated DNA ($3 \cdot 10^{-5}$-10^{-6} erg/mm^2; 254 mμ) were produced in rabbits by immunization, as described by Plescia, Braun and Palczuk. These sera did not react directly with native, denatured, single-strand DNA nor with UV-irradiated RNA. Antibodies induced by UV-irradiated DNA (dose rate, 10^6 erg/mm^2) began to react with DNA exposed to UV at a dose rate of $3 \cdot 10^5$ erg/mm^2.

In view of the wide acceptance of the protein nature of the regulators of /483 gene activity, considerable interest attaches to question of the ability of repressor proteins to "recognize" segments of a DNA molecule with specific nucleotide sequences. At the present time, only two proteins having such properties are known [1].

Antibodies which react with DNA may prove to be a promising model for the investigation of this question. Once the problem of obtaining antibodies which react with DNA was experimentally solved [2], it became possible to attempt to induce synthesis of antibodies specific for DNA that had undergone various modifications, and thereby obtain new information on the question of the ability of proteins to interact with specific segments of a DNA molecule.

At the present, we know of only one investigation devoted to this question. Seaman et al. [3] succeeded in obtaining antibodies which react with T4 phage DNA that had been exposed to visible light in the presence of methylene blue. Up to 50% of the total guanine in this DNA was destroyed. The antibodies reacted specifically with the photo-oxidized products.

The present investigation studied the ability of UV-irradiated DNA to induce, by immunization, the formation of antibodies which react specifically with those segments of the molecule that had been changed by exposure to this type of radiation. As is known, exposure to UV affects mainly the pyrimidines.

Methodology

Preparations of DNA from calf thymus were isolated according to the method of Kay et al. [4] with additional deproteinization by Sevag's method [5]; bacterial DNA was isolated by Marmur's method [6]. The preparations contained no

*Numbers in the margin indicate pagination in the foreign text.

N.B.: Commas should be interpreted as decimal points in all material that has been reproduced directly from the original foreign document.

more than 2% protein. The purity of the DNA preparations was established by methods based on study of the hypochromatic change in the presence of formaldehyde [7]. RNA was obtained from rat liver by Georgiyev's method [8].

Denatured DNA was obtained by boiling a solution of nucleic acid in a standard salt solution (0.15 M NaCl + 0.015 M trisodium citrate) for ten minutes with subsequent cooling [9].

DNA denatured in the presence of formaldehyde (single-strand DNA) was obtained as follows: A solution of DNA (450 μg/ml) was heated for ten minutes at 100° in the presence of 1.3% formaldehyde, which prevented spiralization of the splitting DNA chains [10].

The nucleic acids (20 μg/ml) were irradiated with a BUV-15 lamp (wavelength, 254 mμ) in 0.01 M NaCl at 25°. The thickness of the DNA solution layer was 0.33 cm.

The radiation dose given to the DNA used for immunization was 10^6 erg/mm^2. The average hypochromatism of the preparation exposed to this dose was determined by boiling with formaldehyde as 5%. Rabbits were immunized by the method of Plescia et al. [2] with UV-irradiated DNA, and with native DNA and single-strand DNA complexed with methylated bovine-serum albumen (MBSA). /484

The components were blended by rapid stirring to obtain a homogeneous suspension of the DNA-MBSA complex.

Ten rabbits were used in the experiment. Four rabbits were immunized with UV-irradiated DNA, two with native DNA, and two with formaldehyde-denatured DNA (single-strand DNA). Freund's stimulant [11] with MBSA was administered to two animals.

The passive hemagglutination reaction (PHR) and the antibody neutralization reaction (ANR) based on it were used to detect antibodies and the characteristics of their interaction with the various types of DNA. Erythrocytes containing UV-irradiated (10^6 erg/mm^2) DNA or formalinized DNA were used in the passive hemagglutination reaction. The method of preparing sensitized erythrocytes has been described earlier [12].

The basic principles of the present antibody neutralization study were as follows: Dilutions of various DNA preparations with concentrations differing by a factor of 2 were prepared. Approximately two serum units were added to each sample dilution. (A serum unit means the smallest amount of serum capable of agglutinating 0.05 ml of a 2.5% suspension of sensitized DNA erythrocytes.) Test erythrocytes were added after 30 minutes. When sufficient DNA was present to bind the antibodies, erythrocyte agglutination did not occur. The amount of the studied DNA preparation required to bind a given quantity of antibodies was established in this manner.

Results and Discussion

In all rabbits immunized with UV-irradiated DNA complexed with MBSA, anti-

bodies were formed which react in the passive hemagglutination reaction using erythrocytes sensitized with irradiated DNA in 1/160 and 1/320 dilutions. The results were negative in the reaction with erythrocytes sensitized with DNA. The sera of rabbits immunized with native DNA were inactive in the PHR with various erythrocytes. The sera of rabbits immunized with single-strand DNA reacted only with erythrocytes sensitized with single-strand DNA in 1/1280 and 1/640 dilutions (Table 1).

TABLE 1. PASSIVE HEMAGGLUTINATION REACTION WITH SERA OF IMMUNIZED ANIMALS.

Animal no.	Antigen used for immunization	Titer	
		erythrocytes sensitized with formalinized DNA	erythrocytes sensitized with irradiated DNA
5	UV-irradiated DNA (dose, 10^6 erg/mm^2) complexed with MBSA and Freund's stimulant	less than 1/10	1/160
6	same	same	1/160
7	same	same	1/320
15	same	same	1/320
8	Freund's stimulant with MBSA	same	less than 1/10
9	same	same	same
11	native DNA complexed with MBSA and Freund's stimulant	same	same
12	same	same	same
13	single-strand DNA complexed with MBSA and Freund's stimulant	1/640	same
14	same	1/1280	same

None of the sera of rabbits immunized with UV-irradiated DNA was neutralized in the ANR by significant quantities (8 μg) of native, denatured formalinized DNA, thymine dimers*, and UV-irradiated (10^6 erg/mm^2) RNA. When UV-irradiated DNA was used, antibody neutralization in a volume of two serum units was accomplished by 0.125 and 0.250 μg of DNA (Table 2).

To show that the change in antigen properties is related to the effect of

*Preparation kindly provided by A. S. Sayenko.

TABLE 2. ANTIBODY NEUTRALIZATION REACTION WITH SERA OF RABBITS IMMUNIZED WITH VARIOUS TYPES OF DNA.

Animal no.	Antigen used for immunization	Neutralizing dose, μg				
		native thymus DNA	dentured thymus DNA	formalinized DNA	irradiated thymus DNA	irradiated DNA from E. coli
5	irradiated DNA complexed with MBSA and Freund's stimulant	Does not neutralize*			0.125	0.125
6	same	same			0.250	0.250
7	same	same			0.125	0.125
15	same	same			0.125	0.125
13	single-strand DNA complexed with MBSA and Freund's stimulant	Does not neutralize	1	0.015	Does not neutralize	
14	same	same	4	0.015	same	

*From here on does not neutralize in an 8-μg dose.

radiation on DNA, and not to the effect on admixed proteins, DNA isolated from E. coli was used for the ANR. In this case, the amount of DNA required to bind the antibodies was the same as when the thymus DNA used to immunize the animals is used for the ANR (Table 2).

The ability of the proteins of immunized animals to interact specifically with DNA damaged by UV radiation supports ANR results obtained using thymus DNA treated with pancreatic DNase before irradiation in a pH 5.9 phosphate buffer containing 0.15 M NaCl and 0.01 M $MgSO_4$ for three hours at 37°). The amount of DNA treated with DNase required to bind the antibodies contained in two serum units is significantly greater than the required amount of untreated DNA (following nuclease treatment, 4 μg, compared to 0.125 μg of untreated DNA).

The ability of DNA irradiated with doses, from 1000 to $1.2 \cdot 10^6$ erg/mm^2 to react with antibodies formed by immunizing animals with irradiated DNA ($1 \cdot 10^6$ erg/mm^2) was studied to explain the effect of radiation dose. The minimum radiation dose producing DNA-antibody interaction was high ($3 \cdot 10^5$ erg/mm^2). The results of this study, which illustrate the dependence of neutralizing ability on radiation dose, are presented in Figure 1. /485

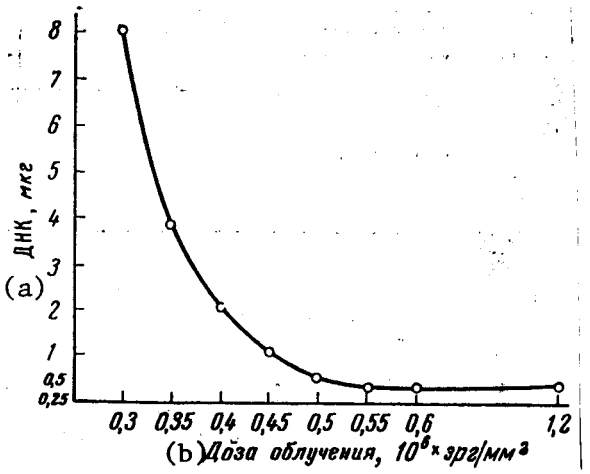

Figure 1. Antibody Neutralization Ability of DNA as a Function of UV Dose. DNA Required to Neutralize Antibodies in a Serum Volume Equivalent to 2 Serum Units is Given in μg on the Ordinate Axis. Key: (a) DNA, μg; (b) radiation dose, 10^6 erg/mm^2.

Thus, the antigen properties of DNA are changed by substantial doses of UV radiation. DNA irradiated with large doses of UV was used in the above described experiments to immunize animals. It remained to be established whether it is possible in principle, to obtain antibodies which react with DNA irradiated with smaller doses. To answer this question, rabbit No. 32 was immunized in a further experiment with an MBSA-DNA complex irradiated by a $3 \cdot 10^5$ erg/mm^2 dose. The antibodies induced in this rabbit failed to react with erythrocytes sensitized with formalinized DNA (titer 1:10), but did agglutinate in a 1:160 titer of erythrocytes sensitized with DNA irradiated with a $3 \cdot 10^5$ erg/mm^2 dose.

The data in Table 3 shows that the serum antibodies interact with DNA damaged by exposure to a $4 \cdot 10^4$ erg/mm^2 dose. Thus, the results obtained for the immunization of animals with DNAs damaged by various UV doses indicate that /486 the determinant groupings in such DNAs are various, and that immunochemical methods can be employed to determine the nature of DNA damage.

TABLE 3. ANTIBODY NEUTRALIZATION BY THE SERUM OF A RABBIT IMMUNIZED WITH DNA IRRADIATED WITH A UV DOSE OF $3 \cdot 10^5$ ERG/MM^2.

Substances used for ANR	Amount of substance (in μg) required to neutralize antibodies (2 serum units)
native DNA	>20
denatured DNA	>10
formalinized DNA,	>10
DNA irradiated with $4 \cdot 10^4$ erg/mm^2	0.25
" " $8 \cdot 10^4$ "	0.12
" " $12 \cdot 10^4$ "	0.03
" " $16 \cdot 10^4$ "	0.015
" " $2 \cdot 10^5$ "	0.0075
" " $3 \cdot 10^5$ "	0.0075

TABLE 3. (Continued)

Substances used for ANR	Amount of substance (in μg) required to neutralize antibodies (2 serum units)
RNA irradiated with $3 \cdot 10^5$ erg/mm^2	>6
polyvinyl sulphate	>300

The data presented show that it is possible to induce the formation of proteins (antibodies) which interact specifically with DNA exposed to UV in doses ranging from $3 \cdot 10^5$ to $1 \cdot 10^6$ erg/mm^2. Antibodies which react with irradiated DNA do not react with native, denatured, nor single-strand DNA. This may be attributed to UV damage to the determinant groups on which antibodies are formed after immunization with denatured DNA. However, this fact may have another explanation. It is known that when animals are immunized with T-even phage DNA, antibodies are formed which react only with those segments of the molecule which contain glycolyzed oxymethylcytosine [13]. This indicates that DNA contains segments on which antibodies primarily form. It is possible that such segments develop in DNA molecules under the effect of ultraviolet radiation.

The appearance of antibodies which react specifically with UV-irradiated DNA demonstrates that the mammalian organism is able to produce proteins which recognize changed segments of the DNA molecule. It is noted that a number of bacteria contain proteins (photoreacting factor) which interact with UV-damaged segments of the molecule [14].

Conclusions

The ability of the mammalian organism to produce antibodies which react specifically with DNA damaged by various doses of ultraviolet radiation is demonstrated. This shows the feasibility of using immunochemical methods to find /487 the nature of damage and modifications of nucleic acid molecules.

In DNA irradiated with various UV doses, the determinant centers on which antibody forms are different and depend on the extent of damage to the nucleic acid.

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